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6. Immunology

- 208 Prostaglandin E2 in exhaled breath condensate in cystic fibrosis**
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Prostaglandin (PG) E2 is an important inflammatory mediator.

We examined pH value and PGE2 concentration in exhaled breath condensate (EBC) in 22 stable CF adults (11 M) aged 25.6±4.1 years (mean ± SD) with FEV1 60.7±21.6% pred. and in 12 (4 M) healthy controls aged 27.6±4.2 years by using ECoScreen condenser. pH was measured without deaeration immediately after collection (Titan pH-meter with CupFET probe). Samples for PGE2 evaluation were stored frozen at -80°C until they were examined with Luminex technology (lower detection limit 35 pg/ml). PGE2 was detectable in all CF patients and healthy controls.

EBC pH was lower in stable CF than in healthy controls (5.50±0.57 vs. 6.09±0.37; p=0.0013) whereas EBC PGE2 was higher (6816±4119 vs. 4228±1613 pg/ml; p=0.027). In stable CF patients, EBC concentration of PGE2 tended to negative correlation with EBC pH (r=-0.344; p=0.122) and was negatively correlated to FEV1 (r=-0.492; p=0.02) in contrast to EBC pH, where positive correlation with FEV1 (r=0.444; p=0.038) was found.

We conclude that EBC PGE2 concentration is higher in stable CF patients than in healthy controls and is negatively correlated to pulmonary function.

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- 210 Lung defense against *Pseudomonas aeruginosa*: the recognition by host cells of either LPS or flagellin is necessary and sufficient**
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Pseudomonas aeruginosa is known to be the main pathogen that infects lungs of cystic fibrosis patients. The innate defense against this pathogen, and many others, is mediated in part through the recognition of specific microbial ligands by Toll-like receptors (TLR), of which there are at least 10 in humans. From *in vivo* experiments, using TLR knockout mice, it is assumed that the control of *P. aeruginosa* occurs through the recognition of LPS and flagellin by TLR2,4 and 5 respectively. In the present study, we investigated *in vitro* the role of these same TLR and ligands, in relation to alveolar macrophages (AM) and respiratory epithelial cells (EC) activation. Cellular responses to *P. aeruginosa* were evaluated in terms of KC, TNF-α, IL-6 and G-CSF secretion, four markers of the innate immune response. AM and EC from WT and knockout mice for TLR2,4 and TLR4,5 were stimulated with the wild-type *P. aeruginosa* or with a mutant devoid of flagellin expression. The results clearly demonstrate that AM and EC recognize *P. aeruginosa* through only two ligand/receptor pairs, *i.e.* TLR4/LPS and TLR5/flagellin. Either one is sufficient to trigger cell activation and when both are missing lung AM and EC are unable to sense the bacterium.

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- 209* Comparison of Inflammatory responses to *Pseudomonas aeruginosa* Lipopolysaccharide isolates compared to *Staphylococcus aureus* Peptidoglycan**

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Cystic Fibrosis (CF) infection by gram-neg and -pos bacteria such as *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA) causes increase Interleukin (IL)-8 release and chronic inflammation. During infection PA undergoes genetic changes in the Lipid A component of the Lipopolysaccharide (LPS).

We aimed to compare the inflammatory response of PA LPS isolates from CF patients differing in disease severity and SA peptidoglycan (PGN) in NON-CF (HTE) and CF (CFTE) tracheal epithelial cells.

CFTE and HTE cells were stimulated with LPS (Sigma: PA, PAO1), PGN (SA) or PA clinical isolates with different Lipid A structure: SE4 (Infant CF), PAK8 (Mild CF), SE22 (Severe CF), Bronch5 (non-CF, bronchiectasis). IL-8 was measured (ELISA). LPS isolates (ng/ml) were more potent than Sigma LPS (ug/ml).

Sigma LPS and PA isolates SE22 and PAK8 showed significant dose dependent IL-8 release in CFTE (all p < 0.002, ANOVA). SE4 and Bronch5 showed no significant response.

IL-8 release differed significantly between both cell lines (100ng/ml): SE22 (p < 0.01) and PAK8 (p < 0.001) induced a higher response in CFTE compared to HTE.

Comparisons between PA (PaK8, Se22) and SA (all 100ng/ml) showed that PA LPS produced a higher inflammatory response than SA PGN in CFTE (all p < 0.02).

The inflammatory response to PA is dependent on strain, cell type and Lipid A modification. To study (patho)physiologically relevant mechanisms of infection an LPS isolate is preferable to commercial LPS. The association between increased response to PA over time, genetic modification and low inflammatory response to SA may explain the PA dominance over SA in CF airways.

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- 211 Role of cytosolic phospholipase A2 in *Pseudomonas aeruginosa*-induced inflammation**

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cPLA2 belonging to a superfamily of enzymes, selectively release arachidonic acid (AA) from membrane PL. AA metabolites play a role in inflammation. *P. aeruginosa* is an opportunistic G⁻ bacteria that infects immuno-compromised and cystic fibroblast patients. This results in high mortality due to its notorious resistance to antibiotics. We postulated that cPLA2 may play a key role in *P. aeruginosa* induced lung inflammation.

Our results showed that in A549 epithelial cells, a WT-strain of *P. aeruginosa* (PAK) increased cPLA2 phosphorylation which reflects its activation, suggesting that cPLA2 may be involved in *P. aeruginosa*-induced inflammation. Both PAK and another *P. aeruginosa* strain, PAO1, induced a time-dependent AA release from A549 cells. The production of prostaglandin E2 (PGE2), a metabolite of AA, was increased markedly by PAK which also induced IL8 synthesis. Meanwhile, PAK induced p38MAPK phosphorylation in a time- and dose-dependent manner. SB2035803, a specific inhibitor of p38MAPK, abolished the effect of PAK on both PGE2 and IL8 synthesis. The expression of cyclooxygenase (COX), which converts AA into PGE2, was increased after PAK stimulation. Finally, the effects of different virulence factors isolated from *P. aeruginosa* were investigated. LPS had no effect on PGE2 release and IL8 synthesis. Flagellin increased IL8 expression but had no effect on PGE2 release. The Δ*fliC* strain, in which the flagellin gene was deleted, had less effect on IL8 synthesis but had similar effect on PGE2, as compared to the PAK strain.

Collectively, our results show that cPLA2 is involved in *P. aeruginosa*-induced inflammation through a process of p38MAPK. However, the virulence factors involved in cPLA2 activation remains to be identified.